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Blue-light receptor in a white mutant of *Physarum polycephalum* mediates inhibition of spherulation and regulation of glucose metabolism

(differentiation/photomorphogenesis/fungal polysaccharide/slime mold)

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ABSTRACT **Blue light induces sporulation of *Physarum polycephalum* macroplasmodia and reversibly inhibits spherulation (sclerotization) of microplasmodia. Illuminated microplasmodia have an abnormal appearance. The photobiological responses of the plasmodia appear to be unaffected by the absence of yellow pigment in the white mutant strain used. Illumination of microplasmodial suspensions with blue light ($\lambda_{max} \approx 465$ nm) results also in an early effect on glucose metabolism: glucose consumption is reversibly inhibited. By using radioactive glucose it was shown that the main products formed are a water-insoluble glucan and the disaccharide trehalose. Inhibition of glucose consumption in the light results in decreased production of these two compounds. Illumination of microplasmodial suspensions also causes a reversible effect on the pH of the medium which is interpreted as a decreased production of a yet unidentified acid from glucose. The action spectrum of the light-induced pH response shows maxima near 390, 465, and 485 nm. It resembles the absorption spectrum of a flavoprotein and confirms the existence of a blue-light receptor in *P. polycephalum* microplasmodia.**

Starving macroplasmodia of the diploid yellow-pigmented myxomycete *Physarum polycephalum* display two alternative pathways of differentiation. In the dark they undergo conversion to resistant encysted structures (sclerotia) which, upon addition of nutrients, revert into plasmodia. In the light, irreversible differentiation into fruiting bodies (sporangia) is induced which is followed by meiosis and allows the initiation of a new life cycle. Germination of the spores liberates mononucleated haploid amoeba which generate a plasmodium by sexual fusion (for reviews see refs. 1–3).

Microplasmodia of *P. polycephalum* that are cultured in suspension rather than in surface culture also differentiate into encysted structures (microsclerotia or spherules) when they are incubated under starvation conditions. Illumination of starving microplasmodia, however, does not result in the formation of spores, a process that apparently requires a dry environment.

The action spectrum of the light induction of sporulation of a closely related species, *P. nudum*, exhibits two broad bands in the blue and the red part of the visible spectrum (4). Green light is antagonistic to blue light. The chemical nature of the photoreceptor and the signal transduction mechanism leading to sporulation are unknown. It has been reported that the injection of illuminated plasmodial pigment extract into *P. polycephalum* plasmodia that have been kept in the dark mimics the action of light by inducing sporulation (5). From these results it was concluded that one of the yellow plasmodial pigments acts as the primary photoreceptor.

In this paper we report on the photobiological properties of a white mutant strain of *P. polycephalum*. Spectroscopic and chromatographic analyses of extracts of white plasmodia have revealed that, within the limit of detection ($<<0.1\%$) the cells contain none of the typical yellow pigments present in the wild type (6). The light induction of sporulation of the white plasmodia, however, appears to be completely unaffected by the absence of yellow pigment.

We assume that the photoreceptor responsible for the induction of sporulation is present in microplasmodia as well as in macroplasmodia. Here we report on the existence of a blue-light receptor in microplasmodia of the white strain. This pigment cannot mediate the induction of sporulation in microplasmodia but it inhibits spherulation, a process that can be regarded as the alternative pathway of differentiation. It is further shown that illumination with blue light has an early effect on glucose metabolism.

MATERIALS AND METHODS

Culture Conditions and Media. The diploid white mutant strain of *P. polycephalum* (LU 897 × LU 898) was originally derived from the amoebal strain LU 688 (7). Microplasmodia were grown on semidefined medium (8). All experiments were performed with 3-day-old cells. For pH measurement the plasmodia were washed three times in 30 mM KCl/3 mM $MgCl_2$/4 mM $CaCl_2$ (pH medium). For assay of metabolites and spherulation the pH medium was buffered with 20 mM potassium citrate at pH 4.7 (spherulation medium).

For all experiments, cells were suspended in the appropriate washing medium at a concentration of 15–20 mm^3 of cells per ml of suspension and D-(+)-glucose (Serva) was added at zero time to give a final concentration of 1 mM. Pretreatment of cells in the described manner is referred to as "standard procedure." Unless stated otherwise, all incubations were carried out in thermostated 15-ml glass cuvettes (23 mm internal diameter) in the dark at 24°C. Cell volume was determined by centrifugation (3 min, 600 × *g*) in 200 mM KCl/4 mM $CaCl_2$/3 mM $MgCl_2$/20 mM potassium citrate, pH 4.7, in centrifuge tubes supplied with a calibrated capillary of 80 mm^3 internal volume.

pH Measurement. Microplasmodia were washed and suspended according to the standard procedure (pH medium/1 mM glucose). Cell suspension (5–8 ml) was stirred in two cuvettes (sample and dark control) and was gently aerated through a 1-μl microcap capillary. At zero time the suspensions were adjusted to pH 6 with 0.1 M NaOH. pH was measured with a pH meter (Knick) supplied with a voltage compensation unit in order to allow magnification of the signal at low pH. The pH in both cuvettes was recorded simultaneously on a two-

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channel recorder (Laumann). Titrations were performed with a microsyringe (Hormuth and Vetter). Photoresponse is expressed as pg of protons consumed per min per mm^3 of cell volume.

Assay of Glucose and Glucose Derivatives. Cell suspension (3–5 ml in sperulation medium/1 mM glucose, standard procedure) as incubated on an orbital shaker (Kuehner) at 250 rpm. Aliquots were taken and added to 0.1 vol of cold 6 M $HClO_4$. After removal of protein by centrifugation (15 min, 16,000 × g) the acid extract was neutralized by adding 0.1 vol of 1 M *N*-ethylmorpholine acetate buffer at pH 6.5 and an equimolar amount of 6 M KOH. Precipitated $KClO_4$ was removed by centrifugation. All manipulations were carried out at 0°C. When necessary, the extract was concentrated by lyophilization. Glucose was assayed as described (9). Polysaccharide hydrolysis (100°C, 15 hr) and reduction of the hydrolysate were performed according to Prehm and Scheid (10). Thin-layer chromatography was carried out on silica gel plates (Merck) with *n*-propanol/NH_3, 60:40 (vol/vol), and ethyl acetate/methanol/glacial acetic acid/water, 30:7.5:7.5:5 (vol/vol), as developing solvents.

High-voltage electrophoresis of sugar alditols was performed in 60 mM sodium borate (pH 9.5) on Cel 400 cellulose plates (Macherey and Nagel) at 40 V/cm (11).

Spherulation Assay. Microplasmodia were incubated in spherulation medium containing 1 mM glucose (standard procedure) and were shaken under sterile conditions at 250 rpm. Percentage of spherulation was calculated by counting both plasmodia and spherules under the light microscope.

Illumination. The light sources were 250-W slide projectors (Noris) or a 150-W xenon lamp (Schoeffel). For action spectra, light was filtered through interference filters (Anders) having a band width of 5–7 nm. For all other incubations under blue light, an FSK 7 filter (Schott, λ_{max} = 450 nm; half-width, 50 nm) was used. This mode of illumination is referred to as "450-nm light." Light intensity (I) was adjusted by using neutral density glass filters (Schott), by regulation of the lamp voltage, or by inserting a variable diaphragm into the light beam. Light intensity was measured by using a calibrated radiometer (EG&G). In all experiments, light was focused on the sample cuvette from the side. The illuminated area was 8 cm^2.

RESULTS

For our studies, plasmodia of the white mutant strain of *P. polycephalum* appeared to be more suitable than plasmodia of the commonly used yellow pigmented strains because the abundant amounts of yellow pigments were likely to interfere with action spectroscopy. The white plasmodia were identical with plasmodia of the yellow strain M_{3CVIII} with respect to their physiological response to light: 100% induction of sporulation (e.g., 10/10 plates) was achieved by continuous illumination with our 450-nm blue light. Also, red light ($\lambda > 600$ nm) was found to be active. Sporulation was not induced by light in the spectral range 515–585 nm and was not observed in the dark. Although at the moment we are not able to present a complete action spectrum of the induction of sporulation in the white mutant strain, we conclude from our observations that the wavelength dependence is likely to be similar to if not identical with the action spectrum of induction obtained for *P. nudum* (4).

A biochemical approach to characterizing the photoreceptor and studying its influence on the differentiation and metabolism of plasmodia seemed to be facilitated by using microplasmodial suspensions rather than macroplasmodia in surface culture. For all experiments described in this report the microplasmodia were suspended in salt medium containing only low concentrations of glucose. These conditions induce the formation of spherules and, in the case of macroplasmodia, make the cells sensitive to light by allowing the photoinduction of sporulation.

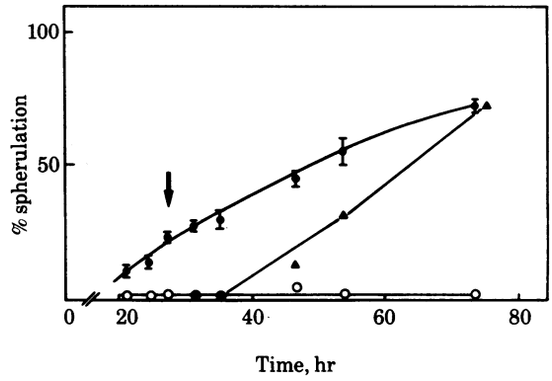

FIG. 1. Inhibition of spherulation by blue light. Cells were suspended at zero time in spherulation medium/1 mM glucose (standard procedure) in eight cuvettes. Four cuvettes (○) were illuminated with 450 nm light (I = 60 nE/sec); the other four served as dark controls (●). % spherulation was determined by counting cells from a 200-μl aliquot under the light microscope. At the time indicated by the arrow illumination of two cuvettes was stopped (▲). At the end of the experiment the number of intact cells per cuvette was found to be identical in all samples. Error bars show SEM (n = 4).

The influence of blue light on the spherulation of white microplasmodia is illustrated in Fig. 1. Spherulation began about 20 hr after initiation of starvation. The spherulation of plasmodia incubated under blue light was completely inhibited. Most of the illuminated plasmodia appeared to be abnormal (Fig. 2). Whereas growing plasmodia have a round or oval shape and a smooth surface, the illuminated cells had an irregular surface with numerous nodular and sometimes needle-like structures.

When the light was turned off, spherulation of the illuminated cells began after a lag phase of about 15 hr and reached the final value of the dark control (Fig. 1). This observation confirms that the unusual shape of the illuminated cells was not caused by partial lysis of plasmodia and that the cells were not damaged by light or by prolonged starvation. The lag phase observed was similar to the lag phase observed in the dark control, suggesting that the light inhibits spherulation at an early stage.

The illuminated cells exhibited protoplasmic streaming which extended into the nodular structures at the cell surface.

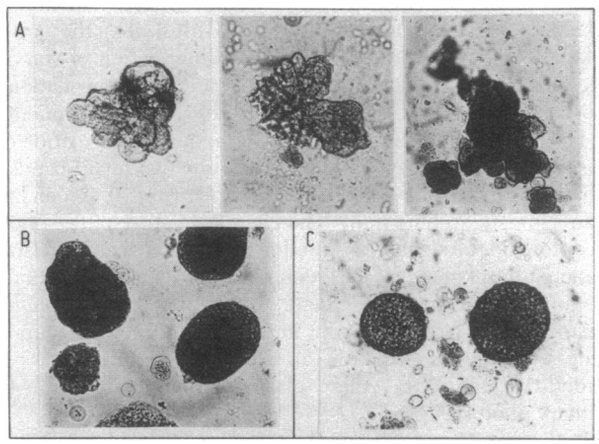

FIG. 2. Influence of light on microplasmodia. Cells were incubated as in Fig. 1. (*A*) Typical cells after 25–30 hr of incubation under illumination with 450-nm light. (*B*) Microplasmodia at zero time. (*C*) Spherules from a culture incubated in the dark for 30 hr. (×140.)

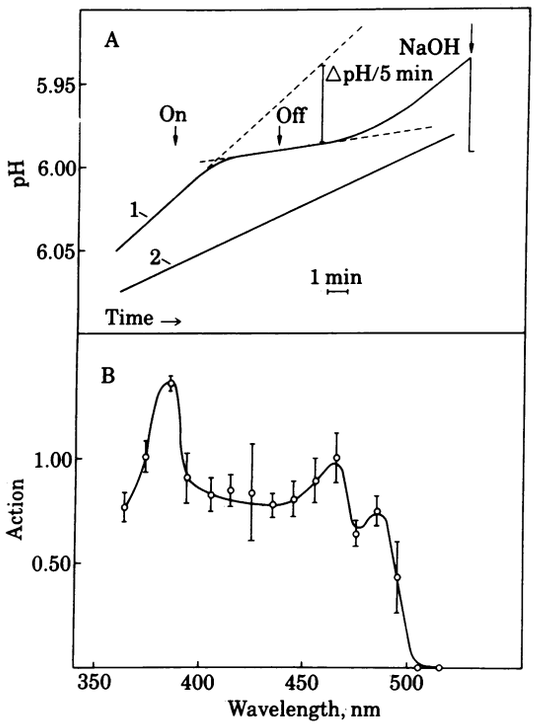

FIG. 3. Light-induced reversible pH change in the medium (*A*) and its action spectrum (*B*). (*A*) Cells were washed and suspended in pH medium/1 mM glucose, pH 6.0, according to the standard procedure. Light (λ_{max} = 456 nm; half-width-5 nm; I = 24 nE/sec) was focused on the sample cuvette (curve 1). The control cuvette (curve 2) was kept in the dark. Initial velocity of light-induced change in proton concentration ($\Delta[H^+]_{hr}$) was quantitated by evaluation of ΔpH as indicated (---) and by titration of the sample. (*B*) Conditions as in *A*. Illumination was through interference filter (5-nm half-width). For all wavelengths, the same light intensity, 1.5 nE/sec, was used. Action was proportional to light intensity and is expressed for each wavelength as $\Delta[H^+]_{hr}$ relative to the reference value obtained with 465-nm light (action = 1). After every illumination (t = 6 min) the sample was kept in the dark for 5 min and was then readjusted to the initial pH value. Error bars show SEM (n = 4–6).

We conclude therefore that these structures do not represent portions of protoplasm separated from the plasmodium by intracellular membranes. Cleavage of the protoplasm by a newly synthesized intracellular membrane system during sporulation has been reported by several authors (12–14). We assume that this process does not take place in the light.

Illumination of the microplasmodial suspension with light of different wavelengths (λ_{max} = 552, 601, and 650 nm with half-width = 30, 45, and 65 nm, respectively; all experiments at an intensity of 60 nE/sec) revealed only a negligible inhibition of sporulation and clearly confirmed a blue-light receptor as the responsible pigment.

The light intensity used in the experiment of Fig. 1 (7.5 nE/sec = 20 W/m^2; 450-nm light) was estimated to be within the range of physiological light concentrations. Bright sunlight was shown to amount to 20–30 W/m^2 when passed through the same 450-nm filter. The unfiltered sunlight, however, certainly provided actinic light in the spectral region around 390 nm (also see the action spectrum in Fig. 3*B*) and will therefore easily saturate the light-dependent inhibition of sporulation.

Daniel (15) has reported that a change in pH of the culture medium of macroplasmodia of *P. polycephalum* is induced by illumination of the cells with white light. He also presented evidence that this light-induced pH change might be related to the induction of sporulation because, from several illuminated macroplasmodia, only those that showed a light-induced pH change differentiated into fruiting bodies. We reinvestigated this reaction in suspensions of microplasmodia of the white strain in order to characterize its wavelength dependence and to find a possible relationship to the light inhibition of sporulation described above.

For this purpose, microplasmodia of the white strain were incubated in pH medium and the suspension was adjusted to pH 6. Under these conditions the plasmodia released an acid into the medium (Fig. 3*A*, curve 2) and in 2–3 hr of incubation lowered the pH to values around 4. Illumination of the suspension with blue light resulted in a reversible inhibition of acidification. (Fig. 3*A*, curve 1). The on and the off responses to light occurred with a lag phase of 1.5–2 min. The initial rate of the pH change could be quantified easily.

The action spectrum of the response to light exhibited maxima near 390, 465, and 485 nm and resembled the absorption spectrum of a flavoprotein (Fig. 3*B*). The dependence of the reaction on light intensity (465-nm light) revealed the existence of two processes. At low light intensity, a linear dependence was observed. This reaction was saturated at values of about 2 nE/sec, the photoresponse at this point amounting to 20 pg of H^+/min per mm^3. At intensities >2 nE/sec, the response did not reach a plateau but increased with a smaller slope (data not shown).

From the type of experiment shown in Fig. 3*A*, no decision can be made as to whether the pH changes observed in the dark and in the light are caused by H^+ transport or reflect net changes in the concentration of an acid. In order to decide between these two alternatives, we incubated microplasmodia in the light and in the dark and measured the pH changes both in the medium (supernatant) and in the homogenate of the cell suspension. The results of a typical experiment are shown in Fig. 4.

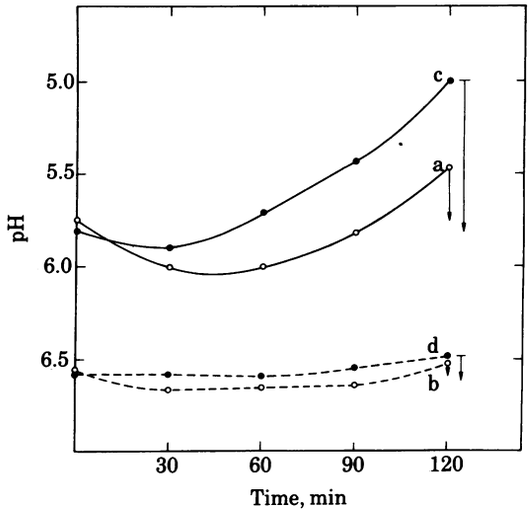

FIG. 4. Inhibition of acid production by light. The time course of the pH change in illuminated cell suspensions (○) and in dark controls (●) was measured in the medium (——) and in the homogenate (– – –). Cells were washed and suspended as in Fig. 3. Incubation was performed in eight cuvettes on an orbital shaker. Illumination of four cuvettes was carried out with 450-nm light (I = 60 nE/sec). For pH measurement 0.5-ml aliquots were taken. Cells were separated from the medium by centrifugation for <1 min, and both fractions were immediately frozen in liquid nitrogen. Homogenates were prepared by recombining pellet and supernatant followed by gentle sonication. Back titration (arrows) of the 120-min samples to the pH value of the corresponding 0-min sample revealed a $\Delta[H^+]$ of 10.8 μM (curve a), 11.6 μM (curve b), 34.5 μM (curve c), and 39.0 μM (curve d), indicating identical changes in H^+ concentration of the dark samples and the light samples, respectively. All data are means (n = 4).

The acidification of the medium in the dark sample correlated with a decrease of the pH in the homogenate. The change in pH was considerably smaller due to the higher buffering capacity of the homogenate relative to the supernatant. However, back titration of the final samples (arrows in Fig. 4) to the pH values of the zero time samples required the same amount of NaOH in both the supernatant and the homogenate.

These results are evidence that the acidification of the medium in the dark is quantitatively explained by the net synthesis and secretion of an acid and not just by the extrusion of protons to the cell exterior. By using the same criteria it can be concluded from the results shown in Fig. 4 that the light-induced pH change is quantitatively explained by the reduced net production of an acid in the light and not by a light-driven H^+ transport.

Both processes—the production of an acid in the dark and its inhibition in the light—depend on the presence of glucose and Ca^{2+} in the medium. They are not observed in glucose-depleted or in Ca^{2+}-depleted cells. Both processes, however, are restored by the addition of glucose and $CaCl_2$, respectively (data not shown). These results suggest that the microplasmodia produce an acid from glucose in a Ca^{2+}-dependent pathway and that blue-light illumination either inhibits the production of the acid or stimulates its consumption. The light-induced pH change in microplasmodial suspensions was also observed when the incubation was carried out under a N_2 atmosphere, indicating that the photoresponse is not related to respiration.

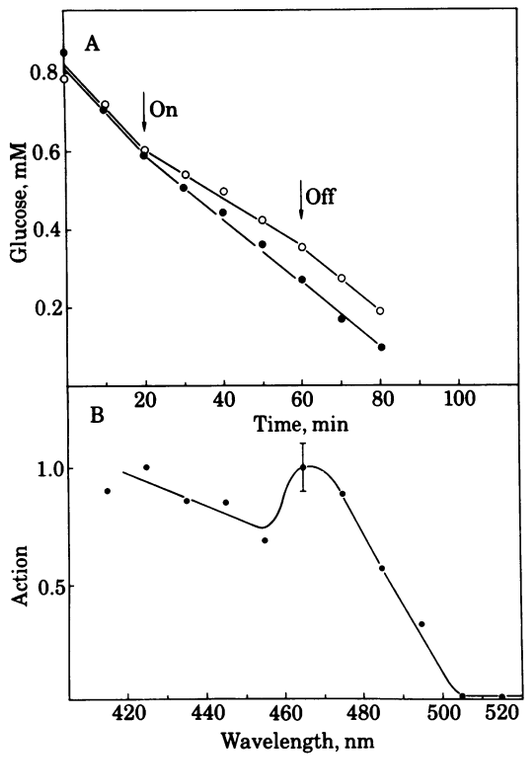

FIG. 5. Reversible light inhibition of glucose consumption (*A*) and its action spectrum (*B*). (*A*) Cells were suspended in spherulation medium/1 mM glucose (standard procedure; four cuvettes). Two cuvettes served as dark controls (●). The sample cuvettes (○) were illuminated with 450-nm light (I = 60 nE/sec). For determination of glucose concentration (9) 100-μl aliquots were taken. All circles represent means of two values. (*B*) Incubation of cells was as in *A*. In each experiment, two cuvettes served as dark controls. Four cuvettes were continuously illuminated; one contained the reference sample (λ_{max} = 465 nm; half-width = 5 nm). Glucose consumption was measured and plotted as in *A*. % inhibition of glucose consumption for each wavelength relative to the dark control was calculated from the graph and divided by the reference value (values represent means from two or three experiments). Error bar shows SEM (n = 11) (reference cuvette). For all wavelengths the same light intensity of 5 nE/sec was used. Action was proportional to light intensity.

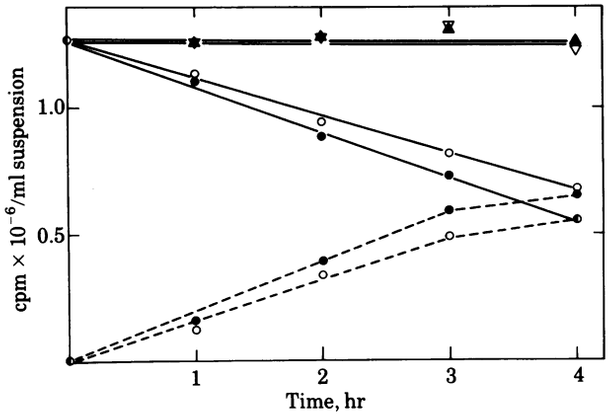

FIG. 6. Light inhibition of glucan formation. Cells were suspended in spherulation medium/1 mM glucose, as in Fig. 5 (eight cuvettes). At zero time, 141 nmol of [*U*-^{14}C]glucose (14 mCi/mmol) was added to each cuvette. Four cuvettes (○) were illuminated with 450-nm light (I = 60 nE/sec). The other four cuvettes served as dark controls (●). Glucan formation (– – –) was measured by measuring the $HClO_4$-precipitable radioactivity from 200-μl aliquots. Less than 5% of the acid-precipitable material was found to be water soluble. Acid-soluble radioactivity was determined in the extract (——). The sums of the corresponding curves illustrate that no radioactivity was lost as CO_2, either in the light (▽) or in the dark (▲).

It has been shown (16) that, during spherulation of *P. polycephalum*, pronounced changes occur in carbohydrate metabolism. Breakdown of glycogen and synthesis of mucopolysaccharide slime as well as of cell wall material have been observed. In order to check this hypothesis we incubated cells in spherulation medium and compared the rates of glucose consumption in the light and in the dark. Illumination with blue light reversibly inhibited glucose breakdown (Fig. 5*A*). The dependence of the photoresponse on light intensity also in this case demonstrated the existence of two light reactions (data not shown). A linear initial process was saturated at about 10 nE/sec. Accumulation of glucose at this point amounted to about 80 nmol/min per mm^3, corresponding to about 20% inhibition of glucose breakdown. At light intensities >10 nE/sec the response did not reach a plateau but increased further with a reduced slope.

The action spectrum of the photoresponse at low light intensities showed a maximum between 460 and 470 nm and a further increase of activity toward lower wavelengths (Fig. 5*B*). We were not able to measure the action spectrum at values <415 nm in order to check for the existence of a second peak at 386 nm as observed in the action spectrum of the light induced pH change (Fig. 3*B*). This was due to the fact that no light source with sufficient intensity was available. On the other hand, the action spectrum was not measurable at light intensities far below 5 nE/sec because the light-induced changes in glucose concentration were small compared to the background. Nevertheless, the similarity of both action spectra in the region 415–505 nm led to the conclusion that the inhibition of glucose consumption and the inhibition of acid production from glucose are mediated by the same photoreceptor.

In order to identify the metabolic pathways inhibited by light, the microplasmodia were incubated with radioactive glucose. Large amounts of radioactivity were incorporated into acid-precipitable ($HClO_4$) material during the first 4 hr of incubation (Fig. 6). When the plasmodia were incubated under

blue-light illumination, radioactivity accumulated in the acid-soluble fraction and a reduced rate of fixation in the insoluble fraction was observed. There was no detectable CO_2 production from glucose under these conditions. The radioactive acid-precipitable material was found to be insoluble in water and in solvents commonly used for lipid extraction but readily dissolved in 0.2% $NaDodSO_4$ or in 1 M NaOH (6). The $NaDodSO_4$-solubilized material did not migrate upon electrophoresis in a 12% polyacrylamide gel and was clearly separated from most of the protein stained by Coomassie blue and from the radioactive glycoprotein bands which did not contribute significantly to the total radioactivity (6). Treatment of the material with various proteases in the presence or absence of $NaDodSO_4$ did not liberate radioactive fragments of low molecular weight. Acid hydrolysis followed by thin-layer chromatography and enzymatic analysis as well as high-voltage electrophoresis of the reduced hydrolysate revealed that glucose was the main radioactive constituent of the polymer. We therefore conclude that the water-insoluble radioactive material is a glucan.

Analysis of the $HClO_4$-soluble cell extracts by thin-layer chromatography revealed that, besides small amounts of ethanol-precipitable mucopolysaccharide, there was only one dominant radioactive soluble product formed from the labeled glucose. Upon analysis by thin-layer chromatography, gas chromatography, and mass spectroscopy this product was found to be identical with the disaccharide trehalose (unpublished data). Preliminary results indicate that trehalose synthesis is also slowed down in the light.

DISCUSSION

We have demonstrated that light controls the differentiation of a white mutant of *P. polycephalum* in macroplasmodia as well as in microplasmodial suspensions. Sporulation of macroplasmodia is induced by illumination with blue or red light. The dependence of induction on wavelength therefore seems to correlate with the action spectrum for the induction of sporulation in *P. nudum* (4). Blue light also inhibits the biological pathway alternative to sporulation—namely, the sclerotization of microplasmodia. We did not observe a significant inhibition of sporulation by red light and therefore regard the photoeffect primarily as a response to blue light.

We have demonstrated that blue light changes the appearance of microplasmodia. The effect was shown to be reversible and could be saturated by physiological light intensities. The illuminated plasmodia appears to be similar to the nodular structures formed from macroplasmodial veins shortly before sporulation occurs. The action spectrum attributed to the blue-light receptor in *P. polycephalum* strongly resembles the adsorption spectrum of a flavoprotein. Similar action spectra have been obtained for various types of blue-light responses in fungi and plants (17, 18).

White plasmodia have been shown to lack the yellow pigments that are present in the wild type (6). We assume that white and yellow plasmodia possess the same photoreceptor system which is responsible for both the light induction of sporulation and the light inhibition of sporulation. We therefore conclude that the abundant amounts of yellow pigment in wild-type strains of *P. polycephalum* do not represent the photoreceptor molecule. Our conclusion is in contrast to the results reported by Wormington and Weaver (5). Their results might be explained by the assumption that a nonidentified component of the injected extract, but not the pigment, was responsible for the induction of sporulation. Nonspecific induction caused by the injection of salts has been reported by the same authors (19). Our conclusion is also supported by the fact that the action spectrum attributed to the blue-light receptor is completely different from the absorption spectra of the yellow pigments (5, 6).

Analysis of the products formed from radioactive glucose during the first 4 hr of incubation did not reveal synthesis of the typical cell wall component galactosamine (20). Instead, the main products were found to be a water-insoluble glucan and the disaccharide trehalose. Both products are likely to be required for sporulation either as a structural component (glucan) or as an energy reservoir for the spherules (21–23). Thus, the inhibition of sporulation by light appears to correlate well with the observed inhibition of synthesis of glucan and trehalose.

An effect of white light on the extracellular pH of *P. polycephalum* plasmodia was reported by Daniel (15) and later was found (24) to be sensitive to gramicidin S. From their experiments the authors concluded that light mediates a H^+/K^+ exchange. Our experiments with the white mutant strain clearly indicate that the effect of light on the extracellular pH of microplasmodial suspensions is not caused by H^+ transport and is quantitatively explained by the decreased production of an acid. The chemical nature of the acid remains to be established. We have presented evidence, however, that it also is made from glucose. At present we regard the light-induced pH response as a valuable tool for obtaining the action spectrum of the physiological blue-light effects in *P. polycephalum*.

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